

# Middlesex University Research Repository

An open access repository of

Middlesex University research

<http://eprints.mdx.ac.uk>

Donner, Erica, Kosjek, Tina, Qualmann, Signe, Kusk, Kresten Ole, Heath, Ester, Revitt, D. Mike, Ledin, Anna and Andersen, Henrik Ramus (2013) Ecotoxicity of carbamazepine and its UV photolysis transformation products. Science of the Total Environment, 443 . pp. 870-876. ISSN 0048-9697 [Article] (doi:10.1016/j.scitotenv.2012.11.059)

Final accepted version (with author's formatting)

This version is available at: <https://eprints.mdx.ac.uk/14565/>

## Copyright:

Middlesex University Research Repository makes the University's research available electronically.

Copyright and moral rights to this work are retained by the author and/or other copyright owners unless otherwise stated. The work is supplied on the understanding that any use for commercial gain is strictly forbidden. A copy may be downloaded for personal, non-commercial, research or study without prior permission and without charge.

Works, including theses and research projects, may not be reproduced in any format or medium, or extensive quotations taken from them, or their content changed in any way, without first obtaining permission in writing from the copyright holder(s). They may not be sold or exploited commercially in any format or medium without the prior written permission of the copyright holder(s).

Full bibliographic details must be given when referring to, or quoting from full items including the author's name, the title of the work, publication details where relevant (place, publisher, date), pagination, and for theses or dissertations the awarding institution, the degree type awarded, and the date of the award.

If you believe that any material held in the repository infringes copyright law, please contact the Repository Team at Middlesex University via the following email address:

[eprints@mdx.ac.uk](mailto:eprints@mdx.ac.uk)

The item will be removed from the repository while any claim is being investigated.

See also repository copyright: re-use policy: <http://eprints.mdx.ac.uk/policies.html#copy>

# **Ecotoxicity of carbamazepine and its UV photolysis transformation products**

**Erica Donner<sup>1,2\*</sup>, Tina Kosjek<sup>3</sup>, Signe Qualmann<sup>4</sup>, Kresten Ole Kusk<sup>4</sup>, Ester Heath<sup>3</sup>, D. Michael Revitt<sup>2</sup>, Anna Ledin<sup>4</sup>, Henrik Rasmus Andersen<sup>4</sup>**

<sup>1</sup> Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Building X, Mawson Lakes Campus, Mawson Lakes, SA-5095 Australia.

<sup>2</sup> Urban Pollution Research Centre, Middlesex University, Hendon Campus, The Burroughs, London NW4 4BT, United Kingdom.

<sup>3</sup> Department of Environmental Sciences, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia.

<sup>4</sup> Department of Environmental Engineering, Technical University of Denmark, B113, DK-2800, Kgs Lyngby, Denmark

**\* Corresponding author:** tel: +61 8 83023624; fax: +61 8 83023057; email: [erica.donner@unisa.edu.au](mailto:erica.donner@unisa.edu.au)

## **ABSTRACT**

Carbamazepine, an anti-epileptic pharmaceutical agent commonly found in wastewater, is highly recalcitrant to standard wastewater treatment practices. This study investigated the mixture toxicity of carbamazepine transformation products formed during UV photolysis using three standard ecotoxicity assays (representing bacteria, algae and crustaceans). UV-treatment of 6 mg L<sup>-1</sup> carbamazepine solution was carried out over a 120 min period and samples were removed periodically over the course of the experiment. Quantification results confirmed the degradation of carbamazepine throughout the treatment period, together with concurrent increases in acridine and acridone concentrations. Ecotoxicity was shown to increase in parallel with carbamazepine degradation indicating that the mixture of degradation products formed was more toxic than the parent compound. In fact, ecotoxicity was still greater than 60 % for all three endpoints even when the carbamazepine concentration had decreased to < 1 % of the starting concentration, and acridine and acridone had decreased to < 10 % of their maximum measured concentrations. Single compound toxicity testing also confirmed the higher toxicity of measured degradation products relative to the parent compound. These results show that transformation products considerably more toxic than carbamazepine itself are likely to be produced during UV treatment of wastewater effluents and/or photo-induced degradation of carbamazepine in natural waters. This study highlights the need to consider mixture toxicity and the formation and persistence of toxicologically relevant transformation products when assessing the environmental risks posed by pharmaceutical compounds.

## **KEYWORDS**

Pharmaceuticals, environment, toxicity, carbamazepine, acridine, acridone, mixture

## INTRODUCTION

There are large knowledge gaps concerning the environmental fate and effects of most active pharmaceutical ingredients (APIs). For regulators, this translates to a high degree of uncertainty about the risks associated with pharmaceutically derived contaminants in the environment (Kümmerer, 2009a, 2009b). The quantities of APIs released to the environment may be relatively low in comparison with other types of pollutants such as pesticides and industrial pollutants, but there is a tendency for their environmental release to be continuous (via wastewater effluents) and the potential for environmental accumulation and/or chronic ecotoxicity has thus been noted (e.g. Besse and Garric, 2008; Fent et al., 2006; Ferrari et al., 2003; Hernando et al., 2006; Escher et al., 2011).

Carbamazepine, an antiepileptic pharmaceutical compound and mood stabilising drug, has attracted particular attention in recent years due to its widespread detection in municipal wastewaters (e.g. Ternes, 1998; Ollers et al., 2001, Falås et al., 2012), surface waters (e.g. Ternes, 1998, Metcalfe et al., 2003; Andreozzi et al., 2003), and drinking waters (e.g. Stackelberg et al., 2004, 2007; Togola and Budzinski, 2008; Heberer et al., 2004). It is also noted to be highly resistant to biodegradation and thus highly recalcitrant under standard biological wastewater treatment conditions (Clara et al., 2005; Kosjek et al., 2009, Falås et al., 2012). Under these circumstances, advanced oxidation processes (AOPs) may offer additional treatment value (von Gunten, 2003; Huber et al., 2005; Macova et al., 2010; Kosjek et al., 2011, Hey et al., 2012; Keen et al., 2012), however complete oxidation and mineralisation is not always achievable and stable transformation products formed during AOPs may also pose environmental risks (Aguerra et al (2005). The stability and ecotoxicity of transformation products formed during treatment and environmental transport are of major importance when assessing the suitability of water treatment options and in determining environmental risks associated with APIs in the environment.

This paper presents the results of research investigating the ecotoxicity of carbamazepine and two of its UV transformation products (Kosjek, 2007), acridine and 9(10H)-acridone. In addition to single compound testing of these compounds using three different standard test organisms (bacteria, algae, and crustacean), an experiment was also conducted to investigate the changes in ecotoxicity occurring during UV treatment of water spiked with 6 mg L<sup>-1</sup> carbamazepine. This experiment, conducted using a bench-top circulating flow UV-reactor system, was designed to compare the ecotoxicity of the initial carbamazepine-spiked solution with that of samples collected during the treatment process. These samples contained mixtures of carbamazepine and its UV-transformation products.

This investigation into the relative toxicity of carbamazepine and its phototransformation products is relevant to future discussions regarding the treatment, control and fate of carbamazepine and carbamazepine-derived contaminants in the environment. Knowledge about carbamazepine's UV-induced degradation pathway is also of interest from the perspective of sunlight-induced photochemical oxidation, as this may be a significant process controlling the gradual ongoing breakdown of carbamazepine released to receiving waters and natural aquatic environments. Indeed, for non biodegradable, non-sorbing APIs such as carbamazepine, sun-induced photodegradation may be the most important long-term process affecting persistence and toxicity in the environment.

This study provides an example of the ‘effect-driven approach’ for determining the risks associated with pollutant transformation products. The ‘effect driven approach’, described by Escher and Fenner (2011) in their paper on advances in environmental risk assessment of transformation products, focuses on ecotoxicity testing of reaction mixtures in which a parent compound is undergoing degradation (e.g. during UV-treatment). This approach facilitates prioritisation of APIs and their transformation products for risk assessment purposes without requiring the prior identification and quantification of individual transformation products. In ‘effect driven’ assessments, compounds are prioritised for transformation product identification and further study if the reaction mixture increases in toxicity in parallel with decreasing parent compound concentration.

## 1. METHODS

### 2.1 Standards, Solvents, and Other Chemicals.

Carbamazepine (99%, CAS 298-46-4) and N-Methyl-N- [tert-butyldimethylsilyl]trifluoroacetimide (MTBSTFA) were purchased from Acros Organics. Acridine (97%, CAS 260-94-6) and 9(10H)-acridone (99%, CAS 578-95-0) were obtained from Sigma-Aldrich.

### 2.2 Chemical analyses

Solid phase extraction (SPE) was used for sample preparation for all chemical analyses. Concentrated solutions (i.e. stock solutions and selected concentration check samples from the acute toxicity test dilution series) were initially diluted as appropriate (dilution factors ranged from 17 to 600) before loading at neutral pH onto Oasis<sup>®</sup> HLB reversed-phase sorbent SPE cartridges (Waters, Corp., Milford, MA, USA). These cartridges were also used for preconcentration of the 150 mL subsamples taken throughout the carbamazepine UV-treatment experiment. SPE cartridges were conditioned with 3 mL of ethyl acetate, 3 mL methanol, and 3 mL of tap water. Sample aliquots were loaded on the SPE columns at a flow-rate of 4–5 mL min<sup>-1</sup>. Each cartridge was then washed with water (3 mL), dried for 30 min under vacuum and eluted with 1 mL acetone, 1 mL of 7/3 ethyl acetate / acetone mixture and 1 mL of ethyl acetate. The combined eluant was evaporated to dryness under a gentle nitrogen stream and reconstituted with 0.5 mL ethyl acetate.

Acridone and carbamazepine were transformed into the tert-butyldimethyl-silyl ether derivatives by adding 30 µL MTBSTFA and maintaining the samples at 60°C for 12 hours. A Varian 3800 gas chromatograph (GC) interfaced with an ion trap Saturn 2000 mass spectrometer (MS) was used for analysis. 10 µL samples were injected (split-splitless) using a PTV injector at 80 °C for 0.30 min before being increased by 200°C/min to 300°C and held for 5 min. A Zebron ZB-5 HT INFERNO 30 m × 0.25 mm × 0.25 µm (Phenomenex) column was used for separation. The GC oven temperature programme was originally held at 80°C for 1 min, then increased by 25°C/min to 225°C and held for 1 min; increased by 1°C/min to 231°C; increased by 10°C/min to 280°C; increased by 45°C/min to 320°C and held at 320°C for a further 3 min. The total runtime was 22.59 min. The mass analyser was operated in electron ionisation (EI) mode, and the following fragment ions were monitored for quantitation: *m/z* 179 for acridine, and *m/z* 252 and *m/z* 193 for the acridone-MTBS and carbamazepine-MTBS derivatives respectively.

For each sample, two parallel subsamples were extracted, derivatised and analysed according to the above protocol. Furthermore, each of these subsamples was injected and analysed twice, with the mean value taken as the relevant concentration. Method performance analyses showed satisfactory linearity ( $r^2 > 0.98$ ) and good repeatability for all three analytes. Blank control samples were also prepared and analysed.

### 2.3 Ecotoxicity testing

Three internationally standardised aquatic ecotoxicity tests (Table 1) were used to investigate the ecotoxicity of carbamazepine, acridine, and acridone. All measurements were conducted in triplicate. The selected ecotoxicity tests used organisms from different trophic levels and included the following short-term toxicity tests:

- inhibition of bioluminescence in the marine bacterium *Vibrio fischeri* (Biotox testing kit; ISO 11348-3, 1998);
- growth inhibition of the green algae *Pseudokirchneriella subcapitata* (ISO 8692, 2004);
- immobilisation of the crustacean *Daphnia magna* Straus (Cladocera, Crustacea) (ISO 6341, 1996).

Table 1: Summary of experimental conditions for bacteria, algae, and daphnia ecotoxicity assays

	<b>Bacteria</b>	<b>Algae</b>	<b>Cladoceran</b>
<i>Test species</i>	<i>Vibrio fischeri</i> ( <i>Photobacterium phosphoreum</i> )	<i>Pseudokirchneriella subcapitata</i> ( <i>Selenastrum capricornatum</i> )	<i>Daphnia magna</i>
<i>Temperature (°C)</i>	15	20	20
<i>Optimal pH</i>	7.0 ± 0.2	7.8 ± 0.2	8 ± 0.3
<i>Light source</i>	-	Light intensity of 10,000 lux supplied by cool white fluorescent tubes	Darkness
<i>Media volume</i>	200 µl/ vial	4 ml/ vial	50 ml/ vial
<i>Number of organisms exposed</i>	Variable	5-10·10 <sup>3</sup> cells/ml	5 juvenile <i>Daphnia</i> per beaker
<i>Aeration</i>	Test vial is left uncovered during the test	Constant air exchange through a hole in the lid of each growth vial	Limited aeration (beakers are covered by a watch glass during the test)
<i>Test duration</i>	30 min	48h	48h
<i>Replicates</i>	3 - 6	3 - 6	2 - 3
<i>Assessment endpoint</i>	Inhibition of bioluminescence	Biomass growth	<i>Daphnia</i> immobilisation
<i>Measurement endpoint</i>	Luminescence	Chlorophyll fluorescence	Number of immobilised daphnia
<i>International Standard Organisation (ISO) Reference</i>	ISO 11348-3, 1998	ISO 8692, 2004	ISO 6341, 1996

■

For the Biotox test, 950  $\mu\text{L}$  of sample solution and 50  $\mu\text{L}$  of *V. fischeri* culture were mixed and bioluminescence was measured after 5, 15 and 30 minutes exposure to the test solution. Algal biomasses were determined using acetone pigment extractions as described by Mayer et al., (1997). For all three ecotoxicity tests, range finding tests were initially conducted for each compound and test species, prior to definitive tests combining ecotoxicity testing and test compound quantification. The aim was to obtain Effective Concentration values (i.e.  $\text{EC}_{10}$  and  $\text{EC}_{50}$ ) for each individual compound. In cases where complete inhibition/immobilisation of the test organism/endpoint did not occur within the solubility limits of the test compound, the assay was re-run with six replicates and LOEC/NOEC values were determined.

The same three assays were also used to monitor changes in ecotoxicity during UV treatment of the 6  $\text{mg L}^{-1}$  carbamazepine solution. All bioassays were conducted under static conditions with no renewal of the test solution. Dissolved oxygen and pH were measured at the beginning and end of the testing period to check compliance with standard test conditions. Quality control tests with potassium dichromate were also performed for all organisms. Furthermore, in addition to standard control samples, DMSO carrier controls were also included.

#### **2.4 Preparation of solutions for ecotoxicity testing**

Stock solutions for ecotoxicity testing of individual compounds were prepared in dimethylsulphoxide (DMSO). An aliquot of each stock solution was then diluted in ultra-pure water (MilliQ) to prepare the top concentration for ecotoxicity testing and further diluted using the appropriate growth medium to prepare the full concentration series for each test. The highest concentrations of solvent in the test solutions were always < 0.1 % of the total volume of test solution. The stock solution for the 6  $\text{mg L}^{-1}$  carbamazepine UV-treatment test was prepared in methanol. The concentration of methanol in the starting solution was 0.1 %.

#### **2.5 UV treatment of carbamazepine and related ecotoxicity assessments**

UV treatment of a 6  $\text{mg L}^{-1}$  carbamazepine solution (prepared with MilliQ water) was conducted using a bench scale UV photoreactor with a circulating flow system. This experiment was carried out in triplicate. The apparatus consisted of a steel container (8 L), a waterpump (85  $\text{L h}^{-1}$ ), and a medium pressure metal-halogen UV lamp (690 W). The UV lamp (Bau 42, Scan Research A/S, Denmark) emitted a polychromatic light ( $\lambda = 185\text{-}400\text{ nm}$ ) with enhanced emission in the relevant range for photochemical treatment (i.e. 190-250 nm). The emission spectrum for this lamp has been previously reported (Kosjek et al., 2009).

The fate and behaviour of carbamazepine and its transformation products were studied over a 120 min irradiation period. A stock solution of 60 mg carbamazepine in 10 mL of methanol was prepared and gradually added to 10 L of MilliQ water to give a 6  $\text{mg L}^{-1}$  carbamazepine solution. This was allowed to circulate through the flow system for 10 minutes prior to the beginning of the test in order to allow for thorough mixing of the test solution. The first 150 mL sample (Time 0) was then taken. Subsequent samples were removed after 2, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes of UV exposure. For each sampling event the lamp was switched off following the predetermined exposure period and the system left to circulate for a further 5 minutes before sampling. The temperature of the test solution increased during treatment, however ice was used to blanket the UV treatment system to

maintain the temperature below 50 °C at all times in order to limit thermal degradation. Carbamazepine, acridine and acridone concentrations were quantified throughout the UV treatment process using the SPE and GC-MS method described above. All samples were stored at 4 °C prior to chemical and ecotoxicological analyses.

## 2.6 Dose-response calculations and other statistical analyses

Dose-response data for the *V. fischeri* bioassays were fitted using a log–logistic distribution, and EC<sub>10</sub>, EC<sub>50</sub> values and their 95 % confidence intervals were calculated according to the method of Barnes et al. (2003). For the *P. subcapitata* bioassays, algal growth rate was calculated from the logarithmic increase in cell density in each individual algae culture, assuming exponential growth during the 48-h duration of the assay. Algal data analysis, including calculation of probit and Weibull dose–response models, EC<sub>50</sub> and EC<sub>10</sub> values and their 95 % confidence intervals was carried out using a Windows-based dose–response regression tool programmed by K.O.Kusk which functions according to the method described in Christensen et al., (2009). *D. magna* bioassay results were analysed using the Toxicity Data Analysis and Database Software (Toxcalc<sup>TM</sup> v5.031), which generates median effective concentrations and their 95% confidence intervals and probit values. All other statistics presented in this paper were calculated using Microsoft Office Excel.

## 2. RESULTS

### 3.1 Ecotoxicity of carbamazepine, acridine and acridone

Acute toxicity data for the individual compounds, expressed either as median effective concentrations (EC<sub>10</sub>/EC<sub>50</sub>) or NOECs and LOECs are presented in Table 2. The results of all three acute toxicity assays showed the parent compound, carbamazepine, to be significantly less toxic than either of the tested transformation products. Furthermore, of the two transformation products tested, acridone was consistently less toxic than acridine across all three assays. Table 3 presents a comprehensive list of acute/short-term toxicity results for aquatic based bioassays that have previously appeared in the literature for these three compounds. As a whole these values support the results presented above.

Table 2: Ecotoxicity testing results for carbamazepine, acridine and acridone (all values in mg l<sup>-1</sup>). Results are based on measured solution concentrations.

Ecotoxicity Assay	Carbamazepine	Acridine	Acridone
Bacteria, <i>Vibrio fischeri</i> (5, 15, 30 min)	NOEC = 5.59 LOEC = 8.16	EC <sub>10</sub> = 0.78 – 0.98 EC <sub>50</sub> = 5.34 - 6.90	NOEC = 0.99 - 2.10 LOEC = 1.44 - 3.07
Algae, <i>Pseudokirchneriella subcapitata</i> (48 h)	EC <sub>10</sub> = 39	EC <sub>10</sub> = 0.11 EC <sub>50</sub> = 0.61	EC <sub>10</sub> = 0.87
Cladoceran, <i>Daphnia magna</i> (48 h)	EC <sub>10</sub> > 30	EC <sub>10</sub> = 0.39 EC <sub>50</sub> = 0.71	EC <sub>10</sub> = 0.82 EC <sub>50</sub> = 1.49



Table 3: Published acute toxicity testing for carbamazepine, acridine, and acridone. The table includes a range of different aquatic species as well as results for benthic invertebrates. EC50/LC50 values relate either to solution concentrations or porewater concentrations as relevant to the particular test. All values are given in mg L<sup>-1</sup>.

<b>Organism, test duration, and ecotoxicological endpoint</b>	<b>Carbamazepine EC<sub>50</sub>/LC<sub>50</sub></b>	<b>Acridine EC<sub>50</sub>/LC<sub>50</sub></b>	<b>Acridone EC<sub>50</sub>/LC<sub>50</sub></b>
Bacteria ( <i>Vibrio fischeri</i> ); 15 min; bioluminescence (Kim et al., 2007)	52.2		
Bacteria ( <i>Vibrio fischeri</i> ); 30 min; bioluminescence (Ferrari et al., 2003)	> 81		
Bacteria ( <i>Vibrio fischeri</i> ); 5, 15, 30 min; bioluminescence (Jos et al., 2003)	64 - 79		
Algae ( <i>Chlorella vulgaris</i> ); 48 h; growth inhibition (Jos et al., 2003)	37		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Kim et al., 2007)	>100		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Ferrari et al., 2003)	> 13.8		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Jos et al., 2003)	98		
Cladoceran ( <i>Daphnia spp.</i> ); 48 h; immobilisation (Cleuvers, 2002)	> 100		
Cladoceran ( <i>Ceriodaphnia dubia</i> ); 48 h; immobilisation (Ferrari et al., 2003)	77.7		
Cnidarian ( <i>Hydra attenuata</i> ); 96 h; tulip phase morphology; (Quinn et al., 2008)	29.4		
Bivalve, Zebra mussel ( <i>Dreissena polymorpha</i> ); 96 h; cell cytotoxicity; haemocytes, gill and digestive gland cells; (Parolini et al., 2011)	5.1 – 6.8		
Algae ( <i>Desmodesmus subspicatus</i> ); 24 h; growth inhibition (Eisentraeger et al., 2008)		2.1	
Algae spp. (7 species tested); 96 h; growth inhibition (Dijkman et al., 1997)		0.08 – 0.79	
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Eisentraeger et al., 2008)		4.6	
Cladoceran ( <i>Daphnia pulex</i> ); 48 h; mortality (first instar) (Parkhurst et al., 1981)		2.3	
Cladoceran ( <i>Daphnia pulex</i> ); 28 d; reproduction (adult) (Parkhurst et al., 1981)		0.8	
Cladoceran ( <i>Daphnia pulex</i> ); 24 h; immobilisation (adult) (Southworth et al., 1978)		1.71	
Benthic invertebrate, midge		0.07	

( <i>Chironomus riparius</i> ); 96 h; survival (first instar larvae) (Bleeker et al., 1998)			
Bivalve, Zebra mussel ( <i>Dreissena polymorpha</i> ); 48 h; filtration rate (Kraak et al., 1997)		0.96	
Calonoid copepod ( <i>Diaptomus clavipes</i> ); 14 h; mortality (first naupliar) (Cooney and Gehrs, 1984)		1.55	
Benthic invertebrate, midge ( <i>Chironomus riparius</i> ); 28 d; survival (emergence) (Paumen et al., 2008)		0.37	1.02
Benthic invertebrate, midge ( <i>Chironomus riparius</i> ); 96h; survival (first instar larvae Bleeker et al (1999).		0.40	> 4.8
Benthic oligochaete ( <i>Chironomus riparius</i> ); 28 d; mortality (Paumen et al., 2009)		0.25	0.75
Benthic oligochaete ( <i>Lumbriculus variegatus</i> ); 28 d; reproduction (Paumen et al., 2009)		0.23	0.1

### 3.2 Carbamazepine UV treatment experiment

UV-treatment of 6 mg L<sup>-1</sup> carbamazepine solution was carried out over a 120 min period and samples were removed periodically over the course of the experiment for ecotoxicity testing. The starting concentration of 6 mg L<sup>-1</sup> is higher than measured environmental concentrations (Lacey *et al.*, (2012), but facilitated the collection of measureable toxicity data from beginning to end of the UV-treatment experiment, even though the concentration of carbamazepine had reached < 1 % of the starting concentration by the end of the experiment. Quantification results confirmed the degradation of carbamazepine throughout the treatment period, together with concurrent increases in acridine and acridone (Figure 1). Toxicity was shown to increase in parallel with these changes, indicating that the mixture of transformation products formed was more toxic than the parent compound by itself (Figures 2, S1, and S2; Table S1).

Figure 1 shows the results of chemical quantification analyses performed on duplicate samples taken during one of the 6 mg L<sup>-1</sup> carbamazepine UV-treatment tests. The concentration of carbamazepine decreased steadily during the UV exposure experiment (i.e. tenfold decrease in concentration within the first 60 min exposure), but was still detectable after 120 minutes. Quantifiable transformation products (i.e. those with commercially available standards) reached a peak level after about 15 minutes exposure and then decreased. Concentration profiles of the two transformation products were both below the detection limit after 120 minutes of UV treatment. Although methanol, a potential radical scavenger, was used as the solvent

Figure 1. Measured concentrations of carbamazepine, acridine, and acridone in samples removed periodically from a 6 mg L<sup>-1</sup> carbamazepine UV-treatment experiment. Measurements were carried out in duplicate and both results are plotted.

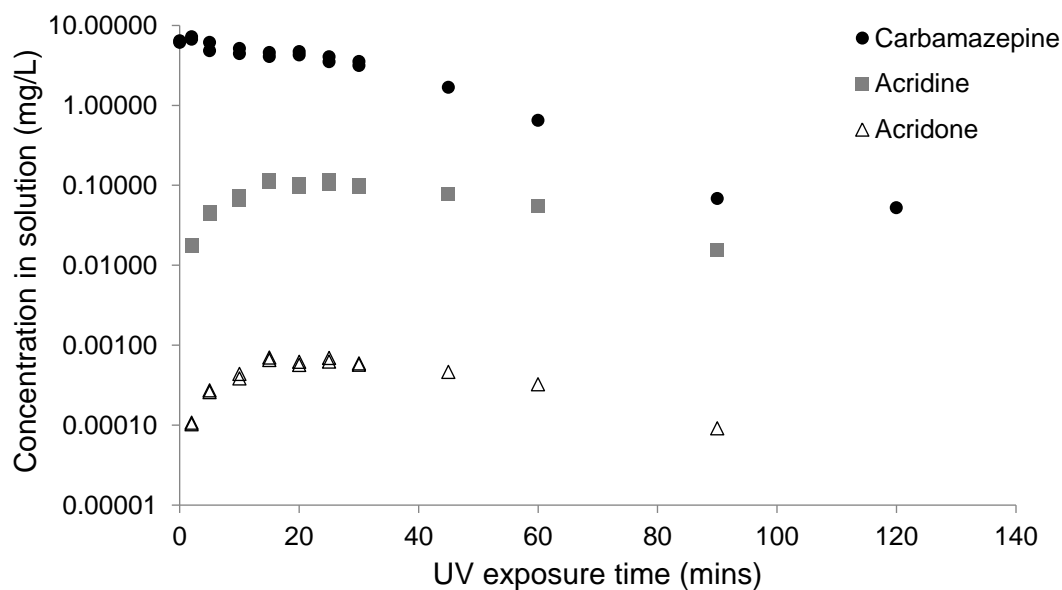
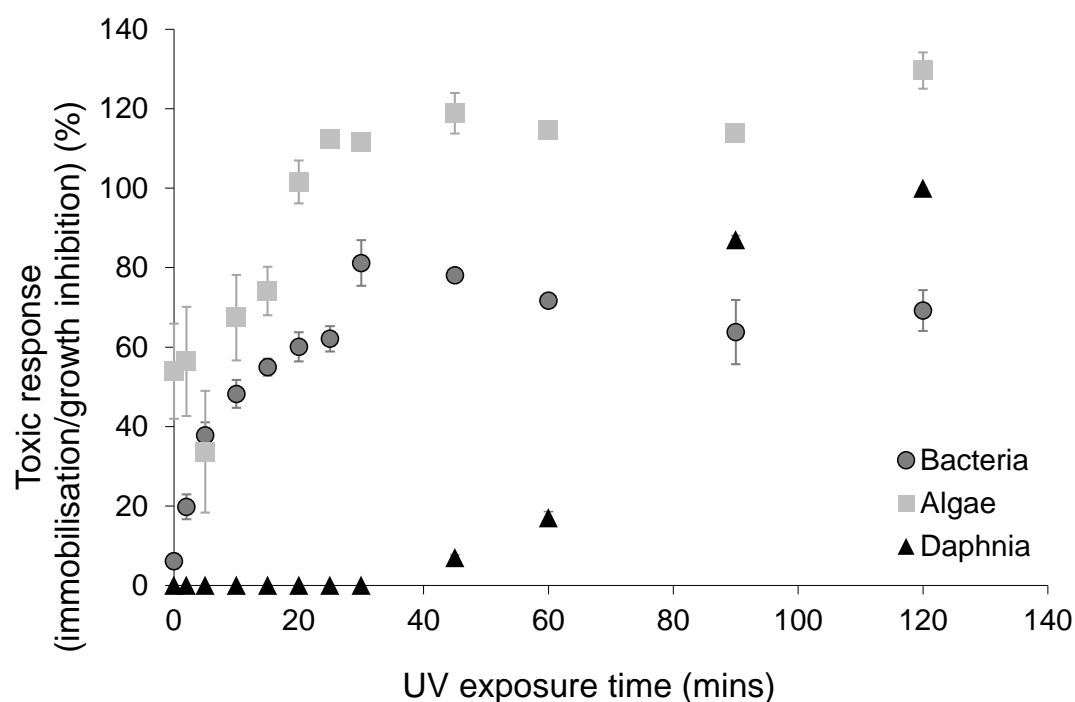


Figure 2. Measured toxic response of *Vibrio fischeri* (inhibition in bioluminescence, 15 min), *Pseudokirchneriella subcapitata* (growth inhibition, 48 h), and *Daphnia magna* (immobilisation, 48 h) exposed to solutions sampled from triplicate runs of the 6 mg L<sup>-1</sup> carbamazepine UV-treatment experiment. Error bars represent the standard error of the mean.



for the carbamazepine stock solution, Kosjek et al. (2009) showed that the concentration of methanol used in this experiment should not have inhibited the decay of carbamazepine or the identified transformation products during treatment. Their investigation used the same benchtop UV-treatment system as the current experiment and showed no significant difference in carbamazepine decay in the presence of 1.2, 2.5 and 6.2 % methanol. As the experimental design for the study reported in this paper included only 0.1 % methanol by volume, the presence of methanol is not believed to have affected the chemical analysis results obtained.

The pH of the carbamazepine UV test solution decreased during treatment from ~ pH 5.8 to pH 3.6. Therefore, it was necessary to amend all samples to a standardised pH prior to toxicity testing in order to prevent pH-induced toxic responses (i.e. hydrogen ion toxicity) from confounding the ecotoxicity results. Samples were therefore amended to the appropriate pH range for each test organism (i.e. ~ pH 7 for *V. fischeri*; pH 7.8 for *D. magna*; pH 8 for *P. subcapitata*). Figure 2 summarises key results from the three ecotoxicity bioassays, and full results for all three organisms are given in Figures S1, S2 and Table S1. The results indicate increasing toxicity over time during UV-treatment in parallel with the formation of carbamazepine degradation products. Peak toxicity to *V. fischeri* occurred around 25 minutes after the onset of UV-treatment when concentrations of acridine and acridone were also at their peaks, however the inhibition of *V. fischeri* bioluminescence remained around 70 % for the remainder of the experimental period, indicating that other transformation products must also be contributing to the ongoing toxic effect. This is also supported by the results of the *P. subcapitata* bioassay presented in Figure S2. In this case growth inhibition reached almost 100 % within 20 minutes of the onset of UV treatment, and greater than 100 % growth inhibition (i.e. cell death) was recorded in subsequent samples. By contrast, *D. magna* was less sensitive to the mixture toxicity of the UV treated carbamazepine solution (Figure 2; Table S1). This bioassay only demonstrated a measurable toxic effect (i.e. immobilisation of the organisms) after 45 minutes of UV treatment, but in keeping with the other bioassays this effect was still evident at the end of the experiment, indicating that toxic transformation products still remained in the solution at the end of the treatment period.

### 3. DISCUSSION

The presented results clearly indicate that the photodegradation products, acridine and acridone, are considerably more toxic than the parent compound carbamazepine across multiple trophic levels. Although the sensitivities of the different test organisms differed, all species showed greater toxicity of the tested photodegradation products compared with the parent compound carbamazepine and this result was further reflected in the increasing toxicity of the carbamazepine spiked water throughout the UV treatment experiment. This is the first time that these compounds have been tested simultaneously under the same experimental conditions and using the same suite of assays, however we note that the results compared favourably with existing acute toxicity data (Table 3).

Recent efforts to quantify concentrations of carbamazepine in surface waters suggest that current environmental levels are well below the concentrations likely to cause acute toxicity to aquatic organisms. For instance, Lacey *et al.*, (2012) referenced ten different studies measuring carbamazepine concentrations in wastewater influents and effluents across Europe and the USA, and reported a maximum effluent concentration

across those studies of  $6.5 \mu\text{g L}^{-1}$ . This is three orders of magnitude smaller than the lowest carbamazepine effect concentration measured in this experiment (*V. fischeri*, LOEC  $8.16 \text{ mg L}^{-1}$ ). However, studies which have investigated chronic/sublethal impacts of pharmaceutical compounds, including carbamazepine, have typically observed physiological effects at much lower contaminant concentrations than the measured threshold concentrations for acute toxicity. For example, Lurling *et al.*, (2006) conducted chronic ecotoxicity tests using *Daphnia pulex* and observed significantly reduced rates of population growth at carbamazepine concentrations of  $200 \mu\text{g L}^{-1}$  due to retardation of juvenile somatic growth and subsequent delays in maturation and time to first reproduction. Similarly, Ferrari *et al.*, (2003) reported carbamazepine NOEC and LOEC values of  $25 \mu\text{g L}^{-1}$  and  $100 \mu\text{g L}^{-1}$  respectively for a chronic 7-day test using reproduction of *Ceriodaphnia dubia* as the endpoint. These studies show that chronic carbamazepine exposure effects may occur at concentrations at least 3 orders of magnitude lower than the  $\text{EC}_{50}$  typically reported for acute *Daphnia* assays (e.g. this paper; Cleuvers, 2002; Jos *et al.*, 2003; Kim *et al.*, 2007), and indicate that, based on estimates of ‘predicted no effect concentrations’ and ‘predicted environmental concentrations’ (Ferrari *et al.*, 2003), carbamazepine poses a potential risk to aquatic environments. Given the current findings regarding the comparative ecotoxicities of carbamazepine and its UV-degradation products, this risk could actually be considerably greater than was previously supposed.

As stated previously, carbamazepine is continually released to the environment at low concentrations due to its ubiquitous presence in wastewater effluents. It is assumed that removal of carbamazepine from surface waters will occur largely via the photodegradation pathway as sorption of carbamazepine is very limited (Ternes *et al.*, 2002; Scheytt *et al.*, 2005; Clara *et al.*, 2004) and it is resistant to biodegradation (Clara *et al.*, 2004; Kosjek *et al.*, 2009). Hence, the formation of stable photodegradation products is not only relevant to wastewater treatment processes, where UV treatment may be implemented to assist with microbial disinfection (e.g. inactivation of *Cryptosporidium*); it is also relevant to the breakdown of carbamazepine in natural river environments. With this in mind it is important to note the recalcitrance of the degradation products formed during this experiment. Even though progressive mineralisation could not be monitored by means of TOC analysis due to the presence of methanol in the spiking solution, the persistent toxicity at the end of the UV-treatment experiment provides evidence that complete mineralisation did not occur. Previous research (Kosjek *et al.*, 2009) has shown that at least six photochemical derivatives are likely to form during the UV degradation of carbamazepine. It should also be noted that Li *et al.*, (2011) reported that oxycarbazepine, a keto analogue of carbamazepine which is increasingly used as a substitute, also degrades to form essentially the same suite of UV-degradation products as carbamazepine. These authors also reported a toxic response in *D. magna* upon exposure to UV-treated oxycarbazepine solutions. By contrast, Rizzo *et al.*, (2009) observed decreasing toxicity when  $5 \text{ mg/L}$  carbamazepine solution was treated by  $\text{TiO}_2$  photocatalysis, but it should be noted that the degradation pathway under that treatment regime (see Calza *et al.*, 2012) would be different to the uncatalysed photolysis pathway relevant in this experiment.

The UV dose applied in the current experiment is higher than that typically used for UV disinfection of water. A typical disinfection dose is equivalent to the UV dose delivered in less than 1 minute in the current experimental setup. Hence, even though

some degradation products may begin to form during UV-disinfection (note that toxic effects were observed in samples taken only 2 min after the onset of UV treatment) UV-disinfection doses are unlikely to significantly degrade carbamazepine. By contrast, if UV were to be purposely used as an end of pipe of treatment to photolyse chemicals the UV dose would be much higher, although not necessarily high enough to remove all toxic degradation products within a feasible timeframe. The feasibility of applying the UV lamp used in this study for degradation of both carbamazepine and other pharmaceuticals and estrogenic chemicals was evaluated in previous studies (Hansen *et al.*, 2007a; Hansen *et al.*, 2007b). Recalculating the doses from that study to the current laboratory setup indicates that 90 % removal of steroid estrogens would be equivalent to 2-5 min treatment (Hansen *et al.*, 2007a), whereas some pharmaceuticals would require the equivalent of 20-40 min UV treatment to be degraded by 90 % (Hansen *et al.*, 2007b).

In this study, mixture toxicity was still high (> 60 % toxicity for all endpoints) at the end of the UV-treatment experiment (Figure 2), even though carbamazepine degradation had reached > 99 % and acridine and acridone concentrations had declined to < 10 % of their peak concentrations. These results highlight the need to further consider the identity, stability, and environmental relevance of recalcitrant carbamazepine degradation products. Researchers have already noted that other pharmaceuticals, such as prednisone (DellaGreca *et al.*, 2003) and diclofenac (Schulze *et al.*, 2010), may also form transformation products more toxic than the parent compound. Failing to account for toxicologically relevant transformation products can easily lead to substances being incorrectly identified as posing negligible environmental risks, when in fact they are significant. However, systematic ecotoxicity testing of treatment transformation products is complicated by the complexity of the relevant degradation pathways and the large numbers of intermediate compounds of varying stability which can be formed (e.g. Agüera *et al.*, 2005; Kosjek *et al.*, 2009; Calza *et al.*, 2012; Li *et al.*, 2012). Moreover, degradation pathways may differ depending on the particular wastewater treatment regime or natural degradation pathway in question (e.g. activated sludge treatment or advanced oxidation vs. sunlight induced photodegradation). It is unrealistic to expect that all relevant compounds can be tested using conventional toxicity testing of individual compounds at multiple trophic levels, especially as the majority of these substances may not be commercially available. Identification, synthesis and isolation of such compounds is extremely time consuming, making experiments such as this, where mixture toxicity of the degradation products together as a whole is assessed, particularly useful for identifying cases where degradation pathway toxicity may be an issue. Further information on effect-driven approaches for assessing the toxicological relevance of transformation products can be found in Escher and Fenner's critical review (2011).

#### **4. CONCLUSION**

The results of this study indicate that degradation products considerably more toxic than carbamazepine itself are likely to be produced as a result of UV treatment of wastewater effluents and/or photo-induced degradation of carbamazepine in natural waters. These findings are clearly relevant to ongoing debates regarding the potential environmental effects of pharmaceutically-derived compounds in the environment, highlighting the need to consider mixture toxicity and the formation and persistence of toxicologically relevant degradation products when assessing environmental risk.

**Acknowledgements**

We thank COST Action 636, Xenobiotics in the Urban Water Cycle for funding E. Donner and T. Kosjek to undertake Short Term Scientific Missions (STSMs) to the Department of Environmental Engineering at the Technical University of Denmark (DTU) where this work was undertaken. This work was also supported by the Swedish MISTRA foundation through the MISTRAPHARMA project. The authors would like to thank Susanne Kruse and Karina Bomholt Henriksen for technical advice and support.

## REFERENCES

Agüera A, Pérez-Estrada LA, Ferrer I, Thurman EM, Malato S, Fernández-Alba AR. Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight. *J. Mass Spectrom* 2005; 40: 908 – 915.

Andreozzi R, Marotta R, Paxéus N. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environments. *Chemosphere* 2003; 50: 1319-1330.

Barnes M, Correll R, Stevens D. A simple spreadsheet for estimating low effect concentrations and associated confidence intervals with logistic dose response curves. Conference paper presented at Society of Environmental Toxicology and Chemistry (SETAC) Asia/Pacific–Australasian Society for Ecotoxicology (ASE) Meeting, Solutions to Pollution 2003, Christchurch, New Zealand, 28 September–1 October 2003.

Besse J-P, Garric J. Human pharmaceuticals in surface waters: Implementation of a prioritization methodology and application to the French situation. *Toxicol. Lett.* 2008; 176: 104–123.

Bleeker EAJ, van der Geest HG, Kraak MHS, de Voogt P, Admiraal W. Comparative ecotoxicity of NPAHs to larvae of the midge *Chironomus riparius*. *Aquat. Toxicol.* 1998; 41: 51 – 62.

Bleeker EAJ, van der Geest HG, Klamer HJC, de Voogt P, Wind E, Kraak MHS. Toxic and genotoxic effects of azaarenes: Isomers and metabolites. *Polycyclic Aromat. Compd.* 1999; 13: 191 – 203.

Calza P, Medana C, Padovano E, Giancotti V, Baiocchi C. Identification of the unknown transformation products derived from clarithromycin and carbamazepine using liquid chromatography/high-resolution mass spectrometry. *Rapid Commun. Mass Spectrom* 2012; 26: 1687 – 1704.

Christensen ER, Kusk KO, Nyholm N. Dose–response regressions for algal growth and similar continuous endpoints: Calculation of effective concentrations. *Environ. Toxicol. Chem.* 2009; 28: 826-835.

Clara M, Strenn B, Kreuzinger N. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of carbamazepine in wastewater treatment and during groundwater infiltration. *Water Research* 2004; 38: 947 – 954.

Clara M, Strenna B, Gans O, Martinez E, Kreuzinger N, Kroissa H. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research* 2005; 39: 4797–4807.

Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicol. Lett.* 2002; 142: 185 – 194.



Cooney JD, Gehrs CW. Effects of temperature, feeding and acridine on development and mortality of eggs and nauplii of *Diaptomus clavipes* Schacht. *Aquat. Toxicol.* 1984; 5: 197 – 209.

Falås P, Andersen HR, Ledin A, Jansen JLC. Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. *Water Sci. Technol.* 2012; 783 – 791.

Hey G, Ledin A, Jansen JLC, Andersen HR. Oxidation of pharmaceuticals by chlorine dioxide in biologically treated wastewater. *Chem. Eng. J.* 2012; 185-186: 236-242.

Hansen K, Andersen HR, Hey T, Ledin A. Photolysis and advanced oxidation treatment of estrogenic chemical in tap water and treated sewage. In: Kalmar Eco-Tech' 07- 10 year anniversary. Sixth international conference on technologies for waste and wastewater treatment - Energy from waste - Remediation of contaminated sites - Emissions related to climate, November 26 - 28, 2007a. Proceedings, vol. 2, pp. 799-807. Kalmar University, Kalmar, Sweden.

Hansen K, Andersen HR, Kosjek T, Heath E, Kaas P, Ledin A, 2007b. Photolysis and advanced oxidation treatment of pharmaceuticals in tap water and treated sewage. In: Kalmar Eco-Tech' 07- 10 year anniversary. Sixth international conference on technologies for waste and wastewater treatment - Energy from waste - Remediation of contaminated sites - Emissions related to climate, November 26 - 28, 2007. Proceedings, vol. 2, pp. 791-798. Kalmar University, Kalmar, Sweden.

DellaGreca M, Iesce MR, Isidori M, Nardelli A, Previtera L, Rubino M. Phototransformation products of tamoxifen by sunlight in water. Toxicity of the drug and its derivatives on aquatic organism. *Chemosphere* 2007; 67: 1933 – 1939.

Dijkman NA, van Vlaardingen PLA, Admiraal WA. Biological variation in sensitivity to N-heterocyclic PAHs: Effects of acridine on seven species of micro-algae. *Environ. Pollut.* 1997; 95: 121 – 126.

Eisentraeger A, Brinkmann C, Hollert H, Sagner A, Tiehm A, Neuwoehner J. Heterocyclic compounds: Toxic effects using algae, daphnids, and the Salmonella/microsome test taking methodological quantitative aspects into account. *Environ. Toxicol. Chem.* 2008; 27: 1590 – 1596.

Escher BI, Baumgartner R, Koller M, Treyer K, Lienert J, McArdell CS, Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater. *Water Research* 2011; 45:75–92.

Escher BI, Fenner K. Recent advances in environmental risk assessment of transformation products. *Environ Sci Technol.* 2011; 45: 3835 – 3847.

Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals, A review. *Aquat. Toxicol.* 2006; 76: 122–159.

Ferrari B, Paxéus N, Lo Giudice R, Pollio A, Garric J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. *Ecotox. Environ. Safe.* 2003; 55: 359–370.

Heberer T, Mechlinski A, Fanck B, Knappe A, Massmann G, Pekdeger A. et al. Field studies on the fate and transport of pharmaceutical residues in bank filtration. *Groundwater Monitoring and Remediation* 2004; 24: 70-77.

Hernando MD, Mezcuca M, Fernández-Alba AR, Barceló D. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta* 2006; 69: 334–342.

Huber MM, Göbel A, Joss A, Hermann N, Löffler D, McArdell CS, Ried A, Siegrist H, Ternes TA, von Gunten U. Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: a pilot study. *Environ. Sci. Technol.* 2005; 39: 4290–4299.

International Standard Organisation. ISO6341. Water quality -- Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) -- Acute toxicity test. International Organization for Standardization, Switzerland; 1996.

International Standard Organisation. ISO11348-3. Water quality -- Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria. International Organization for Standardization, Switzerland; 1998.

International Standard Organisation. ISO8692. Water quality -- Freshwater algal growth inhibition test with unicellular green algae. International Organisation for Standardisation, Switzerland; 2004.

Jos A, Repetto G, Rios JC, Hazen MJ, Molero ML, del Peso A, Salguero M, Fernandez-Freire P, Perez-Martin JM, Camean A. Ecotoxicological evaluation of carbamazepine using six different model systems with eighteen endpoints. *Toxicol. in Vitro* 2003; 17: 525-532.

Keen OS, Baik S, Linden KG, Aga DS, Love NG. Enhanced biodegradation of carbamazepine after UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation. *Environ. Sci. Technol.* 2012; 46: 6222 – 6227.

Kosjek T, Heath E, Kompare B. Removal of pharmaceutical residues in a pilot wastewater treatment plant. *Anal. Bioanal. Chem.* 2007; 387: 1379-1387.

Kosjek T, Andersen HR, Kompare B, Ledin A, Heath E. Fate of carbamazepine during water treatment. *Environ. Sci. Technol.* 2009; 43: 6256-6261.

Kosjek T, Perko S, Zupanc M, Zanoški HM, Landeka DT, Žigon D, Kompare B, Heath E. Environmental occurrence, fate and transformation of benzodiazepines in water treatment. *Water Research* 2011; 46: 355-368.

Kraak MHS, Ainscough C, Fernandez A, van Vlaardingen PLA, de Voogt P, Admiral WA. Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: effects and metabolism. *Aquat. Toxicol.* 1997; 37: 9-20.

Kwon JW, Armbrust KL. Degradation of citalopram by simulated sunlight. *Environ. Toxicol. Chem.* 2005; 24: 1618-1623.

Kümmerer K. The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. *J. Environ. Manage.* 2009a; 90: 2354 – 2366.

Kümmerer K. Antibiotics in the aquatic environment – A review – Part I. *Chemosphere*; 2009; 75: 417 – 434.

Lacey C, Basha S, Morrissey A, Tobin JM. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. *Environ. Monit. Assess.* 2012; 184: 1049-1062.

Li Z, Fenet H, Gomez E, Chiron S. Transformation of the antiepileptic drug oxycarbamazepine upon different water disinfection processes. *Water Research* 2011; 45: 1587 – 1596.

Lürling M, Sargant E, Roessink I. Life-history consequences for *Daphnia pulex* exposed to pharmaceutical carbamazepine. *Environ. Toxicol.* 2006; 21: 172 – 180.

Macova M, Escher BI, Reungoat J, Carswell S, Chue KL, Keller J, Mueller JF. Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Research* 2010; 44: 477–492.

Mayer P, Cuhel R, Nyholm N. A simple in vitro fluorescence method for biomass measurements in algal growth inhibition tests. *Water Research* 1997; 31: 2525-2531.

Metcalf CD, Miao XS, Koenig BG, Struger J. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower great lakes, Canada. *Environ. Toxicol. Chem.* 2003; 22: 2881-2889.

Ollers S, Singer HP, Fassler P, Muller SR. Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at the low ng/l level in surface and waste water. *Journal Chromatogr. A.* 2001; 911: 225-234.

Parolini M, Quinn B, Binelli A, Provini A. Cytotoxicity assessment of four pharmaceutical compounds on the zebra mussel (*Dreissena polymorpha*) haemocytes, gill and digestive gland primary cell cultures. *Chemosphere* 2011; 84: 91-100.

Parkhurst BR, Bradshaw AS, Forte JL, Wright GP. The chronic toxicity to *Daphnia magna* of acridine, a representative azaarene present in synthetic fossil fuel products and wastewaters. *Environ. Pollut.* 1981; 24: 21 – 30.

Paumen ML, Borgman E, Kraak MHS, van Gestel CAM, Admiraal W, Life cycle responses of the midge *Chironomus riparius* to polycyclic aromatic compound exposure. *Environ. Pollut.* 2008; 152: 225 – 232.

Paumen ML, de Voogt P, van Gestel CAM, Kraak MHS. Comparative chronic toxicity of homo- and heterocyclic aromatic compounds to benthic and terrestrial invertebrates: Generalisations and exceptions. *Sci. Total Environ.* 2009; 407: 4605 – 4609.

Quinn B, Gagne F, Blaise C. An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarians, *Hydra attenuate*. *Sci. Total Environ.* 2008; 389: 306 – 314.

Rizzo L, Meric S, Guida M, Kassinos D, Belgiorno V. Heterogenous photocatalytic degradation kinetics and detoxification of an urban wastewater treatment plant effluent contaminated with pharmaceuticals. *Water Research* 2009; 43: 4070 – 4078.

Scheytt T, Mersmann P, Lindstadt R, Heberer T. Determination of sorption coefficients of pharmaceutically active substances carbamazepine, diclofenac, and ibuprofen, in sandy sediments. *Chemosphere.* 2005; 60: 245 – 253.

Schulze T, Weiss S, Schymanski E, von der Ohe PC, Schmitt-Jansen M, Altenburger R, Streck G, Brack W. Identification of a phytotoxic photo-transformation product of diclofenac using effect-directed analysis. *Environ. Pollut.* 2010; 1461 – 1466.

Southworth GR, Beauchamp JJ, Schmieder PK. Bioaccumulation potential and acute toxicity of synthetic fuel effluents in freshwater biota: azaarenes. *Environ. Sci. Technol.* 1978; 12: 1062 – 1066.

Stackelberg PE, Furlong ET, Meyer MT, Zaugg SD, Hendersond AK, Reissmand DB,. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking water treatment plant. *Sci. Total Environ.* 2004; 329: 99-113.

Stackelberg PE, Gibbs J, Furlong ET, Meyer MT, Zaugg SD, Lippincott RL. Efficiency of conventional drinking water treatment processes in removal of pharmaceuticals and other organic compounds. *Sci. Total Environ.* 2007; 377: 255-272.

Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. *Water Research.* 1998; 32: 3245-3260.

Ternes TA, Meisenheimer M, McDowell D, Sacher F, Brauch HJ, Haist-Guide B, Preuss G, Wilme U, Zulei-Seibert N. Removal of pharmaceuticals during drinking water treatment. *Environ. Sci. Technol.* 2002; 36: 3855 – 3863.

Togola A, Budzinski H. Multi-residue analysis of pharmaceutical compounds in aqueous samples. *Journal Chromatogr. A.* 2008; 1177: 150-158.

von Gunten U. Ozonation of drinking water: part I. Oxidation kinetics and product formation. *Water Research*. 2003; 37: 1443–1467.

## SUPPLEMENTARY MATERIAL

Table S1: Published acute toxicity testing for carbamazepine, acridine, and acridone. The table includes a range of different aquatic species as well as results for benthic invertebrates. EC50/LC50 values relate either to solution concentrations or porewater concentrations as relevant to the particular test. All values are given in mg L<sup>-1</sup>.

Organism, test duration, and ecotoxicological endpoint	Carbamazepine EC <sub>50</sub> /LC <sub>50</sub>	Acridine EC <sub>50</sub> /LC <sub>50</sub>	Acridone EC <sub>50</sub> /LC <sub>50</sub>
Bacteria ( <i>Vibrio fischeri</i> ); 15 min; bioluminescence (Kim et al., 2007)	52.2		
Bacteria ( <i>Vibrio fischeri</i> ); 30 min; bioluminescence (Ferrari et al., 2003)	> 81		
Bacteria ( <i>Vibrio fischeri</i> ); 5, 15, 30 min; bioluminescence (Jos et al., 2003)	64 - 79		
Algae ( <i>Chlorella vulgaris</i> ); 48 h; growth inhibition (Jos et al., 2003)	37		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Kim et al., 2007)	>100		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Ferrari et al., 2003)	> 13.8		
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Jos et al., 2003)	98		
Cladoceran ( <i>Daphnia spp.</i> ); 48 h; immobilisation (Cleuvers, 2002)	> 100		
Cladoceran ( <i>Ceriodaphnia dubia</i> ); 48 h; immobilisation (Ferrari et al., 2003)	77.7		
Cnidarian ( <i>Hydra attenuata</i> ); 96 h; tulip phase morphology; (Quinn et al., 2008)	29.4		
Bivalve, Zebra mussel ( <i>Dreissena polymorpha</i> ); 96 h; cell cytotoxicity; haemocytes, gill and digestive gland cells; (Parolini et al., 2011)	5.1 – 6.8		
Algae ( <i>Desmodesmus subspicatus</i> ); 24 h; growth inhibition (Eisentraeger et al., 2008)		2.1	
Algae spp. (7 species tested); 96 h; growth inhibition (Dijkman et al., 1997)		0.08 – 0.79	
Cladoceran ( <i>Daphnia magna</i> ); 48 h; immobilisation (Eisentraeger et al., 2008)		4.6	
Cladoceran ( <i>Daphnia pulex</i> ); 48 h; mortality (first instar) (Parkhurst et al., 1981)		2.3	
Cladoceran ( <i>Daphnia pulex</i> ); 28 d; reproduction (adult) (Parkhurst et al., 1981)		0.8	

Cladoceran ( <i>Daphnia pulex</i> ); 24 h; immobilisation (adult) (Southworth et al., 1978)		1.71	
Benthic invertebrate, midge ( <i>Chironomus riparius</i> ); 96 h; survival (first instar larvae) (Bleeker et al., 1998)		0.07	
Bivalve, Zebra mussel ( <i>Dreissena polymorpha</i> ); 48 h; filtration rate (Kraak et al., 1997)		0.96	
Calonoid copepod ( <i>Diaptomus clavipes</i> ); 14 h; mortality (first naupliar) (Cooney and Gehrs, 1984)		1.55	
Benthic invertebrate, midge ( <i>Chironomus riparius</i> ); 28 d; survival (emergence) (Paumen et al., 2008)		0.37	1.02
Benthic invertebrate, midge ( <i>Chironomus riparius</i> ); 96h; survival (first instar larvae Bleeker et al (1999).		0.40	> 4.8
Benthic oligochaete ( <i>Chironomus riparius</i> ); 28 d; mortality (Paumen et al., 2009)		0.25	0.75
Benthic oligochaete ( <i>Lumbriculus variegatus</i> ); 28 d; reproduction (Paumen et al., 2009)		0.23	0.1

Table S2. Mean immobility of *Daphnia magna* exposed to solutions sampled from the 6 mg L<sup>-1</sup> carbamazepine UV-treatment experiments. Standard errors are given in brackets.

Treatment Time (min)	Mean immobility after 24 h exposure (%)	Mean immobility after 48 h exposure (%)
0	0 (0.0)	0 (0.0)
2	0 (0.0)	0 (0.0)
5	0 (0.0)	0 (0.0)
10	0 (0.0)	0 (0.0)
15	0 (0.0)	0 (0.0)
20	0 (0.0)	0 (0.0)
25	0 (0.0)	0 (0.0)
30	0 (0.0)	0 (0.0)
45	0 (0.0)	7 (0.7)
60	11 (0.9)	17 (1.6)
90	17 (1.2)	87 (1.1)
120	49 (1.4)	100 (0.0)

Figure S1. Mean inhibition in bioluminescence of *Vibrio fischeri* exposed to solutions sampled from triplicate runs of the 6 mg L<sup>-1</sup> carbamazepine UV-treatment experiment. Bioluminescence was measured after 5, 15 and 30 minutes exposure to the sample solutions. Error bars represent the standard error of the mean.

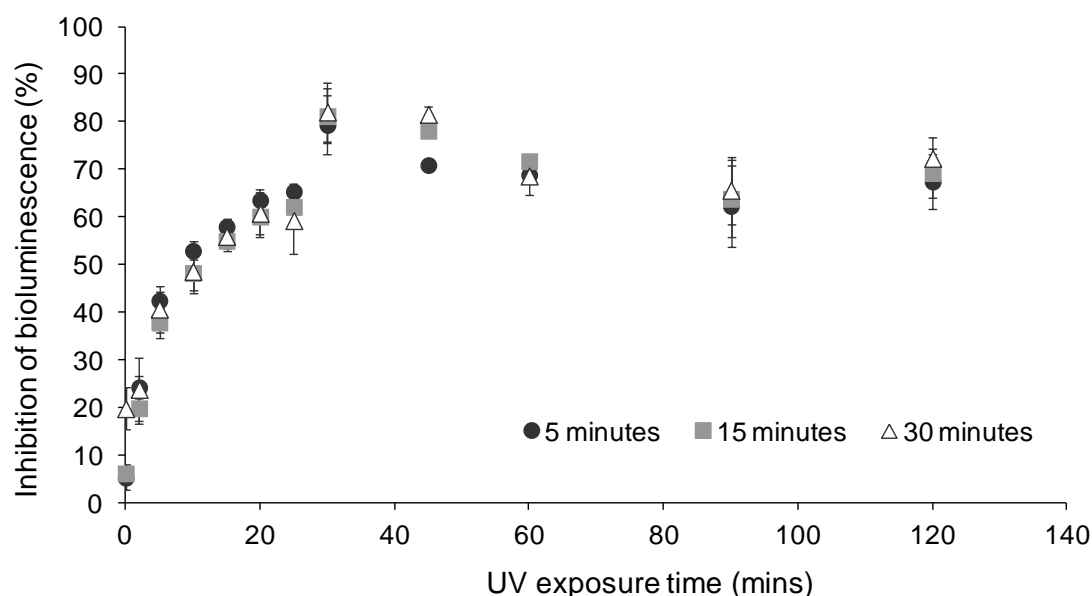


Figure S2. Mean inhibition in growth rate of *Pseudokirchneriella subcapitata* exposed to solutions sampled from the 6 mg L<sup>-1</sup> carbamazepine UV-treatment experiment. Cell density was measured after 0, 24 and 48 h exposure to the sample solutions and the mean of three replicate samples is presented for each exposure time. Error bars represent the standard error of the mean.

